Reply and Amendment U.S. Serial No. 09/127,738

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for a time period sufficient to produce a culture having a compact multilayer like appearance whereby said culturing is performed in the absence of a feeder layer; and

(iii) identifying EG cells contained therein.

Comp

(Twice Amended) An improved method of producing chimeric avians which

comprises:

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(i) isolating primordial germ cells (PGCs) from an avian;

(ii) maintaining such PGCs in a tissue culture medium containing at least the following growth factors;

(1) leukemia inhibitory factor (LIF),

(2) basic fibroblast growth factor (bFGF),

(3) stem cell factor (SCF) and

(4) insulin-like growth factor (IGF)

for a sufficient time to produce embryonic germ (EG) cells whereby said culture is maintained in the absence of a feeder layer;

(iii) transferring said EG cells into a recipient avian embryo; and

(iv) selecting for chimeric avians which have the desired EG phenotype.

See the attached Appendix for the changes made to effect the above claim(s).



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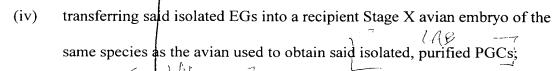
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Please add the following new Claims 25-30: 25. An improved method of producing germline chimeric avians which comprises: (i) isolating primordial germ cells (PGCs) from a Stage XII-XIV avian embryo; 1 Harrist 1.5 maintaining such PGCs in a tissue culture medium containing at least the (ii) following growth factors; leukemia inhibitory factor (LIF), (1) (2) basic fibroblast growth factor (bFGF), (3) stem cell factor (SCF) and (4)insulin-like growth factor (IGF); transferring said PGCs into a Stage XII-XIV recipient avian embryo; and - freducing germany dance aureis selecting for germline chimeric avians which have the desired PGC phenoty 26. An improved method of producing germline or somatic cell chimeric avians which comprises: (i) isolating primordial germ cells (PGCs) from a Stage XII-XIV avian embryo; maintaining such PGCs in a tissue culture medium containing at least the (ii) following growth factors (1) leukemia inhibitory factor (LIF), (2) basic fibroblast growth factor (bFGF), (3) stem cell factor (SCF) and (4) insulin-like growth factor (IGF), for a sufficient time to produce embryonic germ (EG) cells; CAB identifying and isolating embryonic germ (EG) cells from said cultured (iii)

population of primordial germ cells

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- (v) allowing said recipient avian to develop into a bird; and
- (vi) selecting for germline or somatic cell chimeric avians that express the PGC phenotype.

7 27. A method for producing avian embryonic germ (EG) cells comprising the following steps:

- (i) isolating a pure population of primordial germ cells (PGCs) from a Stage XII-XIV avian embryo;
- (ii) culturing said pure population of PGCs for a period of at least fourteen days in tissue culture in the absence of a feeder layer sufficient to produce a culture having a compact multilayer like appearance; and
- (iii) identifying the EG cells contained therein.
- 28. A method for producing chimeric avians comprising:
- (i) isolating a pure population of primordial germ cells (PGCs) from a Stage XII-XIV avian embryo;
- (ii) culturing said pure population of PGCs for a period of at least fourteen days in tissue culture;
- transferring said purified PGCs into a recipient avian embryo of the same species as the avian used to obtain said isolated, purified PGCs;
- (iv) allowing said recipient avian to develop into a bird; and
- (v) selecting for chimeric avians that express the PGC phenotype.

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- 29. A method for producing germline chimeric avians comprising:
- (i) isolating a pure population of primordial germ cells (PGCs) from a Stage XII-XIV avian embryo;
- (ii) culturing said pure population of PGCs for a period of at least fourteen days in tissue culture;
- transferring said purified PGCs into a recipient Stage XII-XIV avian embryo of the same species as the avian used to obtain said isolated, purified PGCs;
- (iv) allowing said recipient avian to develop into a bird; and
- (v) selecting for germline chimeric avians that express the PGC phenotype.
- 30. (New) A method for producing germline or somatic cell chimeric avians comprising:
 - (i) isolating a pure population of primordial germ cells (PGCs) from a Stage XII-XIV avian embryo;
 - (ii) culturing said pure population of PGCs for a period of at least fourteen days in tissue culture;
 - (iii) identifying and isolating embryonic germ (EG) cells from said cultured population of primordial germ cells;
 - (iv) transferring said isolated EGs into a recipient Stage X avian embryo of the same species as the avian used to obtain said isolated, purified PGCs;
 - (iv) allowing said recipient avian to develop into a bird; and
 - (v) selecting for germline or somatic cell chimeric avians that express the PGC phenotype

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